Development of Protein Profile Technology to Evaluate Ecological Effects of Environmental Chemicals Using a Small Fish Model

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This research is designed to identify endocrine-mediated effects using rapid high-throughput protein fingerprinting techniques to provide EPA with predictive tools for chemical screening and prioritization and enhanced interpretation of exposure, hazard identification, and dose-response information. Current endocrine testing methods are animal intensive and lack the rapid throughput necessary to screen the large number (>80,000) of chemicals required under FQPA and SDWAA. As part of a tiered approach, new molecular techniques are being developed to assist EPA in evaluating and prioritizing chemicals for testing. Ultimately, a tiered testing approach will be used to screen and assay these thousands of compounds. *In silico* QSAR models will be employed to identify compounds with the greatest likelihood of disrupting endocrine systems through known, modeled endocrine pathways. The small number of compounds identified by the computer models as potential EDCs may then be subjected to *in vivo* screening tests, such as presented in this task, to assist in prioritization of chemicals before more costly Tier 2 definitive testing is initiated. Recently, a cost-effective method has been developed to simultaneously analyze large numbers of proteins in biological samples to provide rapid tissue-specific protein expression profiles. The Gulf Ecology Division, in partnership with the American Chemical Council, are currently exploring the utility of fish plasma protein profiling as a rapid and cost-effective means to screen large chemical inventories for pathway-specific toxicity in vertebrate species. As proof of concept, GED scientists are using a Ciphergen ProteinChip® Biomarker System that employs a Surface Enhanced Laser Desorption/Ionization Time-of-Flight Mass Spectrometer (SELDI-TOF MS) to examine protein patterns associated with exposure to EDCs, specifically, estrogenic compounds. Plasma samples from control and treated fish are applied to ProteinChip arrays, producing spectral fingerprints that are characteristic for a specific estrogenic pathway as determined using pattern recognition software. After establishing a definitive baseline with the native ligand, 17β-estradiol, other compounds representative of specific modes of estrogenic toxicity, including receptor-mediated and non-receptor-mediated pathways, will be evaluated. Specific protein profiles generated for known estrogenic pathways will then be incorporated into a predictive estrogen-responsive pattern recognition model. Chemicals with unknown properties or tissues supplied from field-collected organisms can be tested and evaluated for estrogenic activity based on comparison with the model. Although the patterns generated will be specific to the species tested, the protocol should be readily transferrable to any species or chemical toxicity mechanism of interest.